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A survey on *Nosema apis* infection in apiaries of Urmia, North-West of Iran

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Abstract

The microsporidium *Nosema apis* is a protozoan parasite specific for the epithelial cells of the ventricles of adult bees. Nosemosis occurs throughout the world. Between February and May 2002-2003, samples were randomly collected twice from 20 apiaries in Urmia region North-West of Iran. During this survey total of 487 hives were examined for infection with *N. apis*. In the first stage of study (February 2002), no infection was seen in hives samples, but in second stage of same year (May 2002) the infection was seen in one apiary with 131 hives. In third and forth stage of study (February and May 2003) the infection was seen in one and 4 apiaries respectively. The results showed the low infection rate in the early months of the first year of study that it was increased in next year. The reason of high infection rate in the second year of the study can be the result of more rainy and cloudy days in this period comparing to the first year. This has caused the hone bees to stay more often in the hives and increase spores excreted and subsequently spore intake were raised.

Keywords: *Nosema apis*, apiaries, Honeybees, Urmia, Iran.

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Introduction

The microsporidium Nosema apis is a protozoan parasite specific for to the epithelial cells of the ventriculus of adult bees; nosemosis occurs worldwide (Matheson, 1996). Infection occurs by the ingestion of spores in the feed (Bailey, 1981 and L'Arrivee, 1965), via trophallaxis (Weiser, 1961) or perhaps after grooming of the body hairs (Bulla, 1977; Fries, 1988 and Morgenthaler, 1939). The polar tube of the spore is turning outward and penetrates the peritrophic matrix of the intestine, particularly in the posterior region of the ventriculus. The sporoplasm passes down the tube and enters the cytoplasm of the epithelial cells, where it reproduces. Autoinfection can also occur. After a short interval, spores develop in large quantities. Infected bees are unable to fly, and sometimes one bee can be infected with up to 500 million spores. The parasite is ubiquitous and multiplies at a specific rate throughout the year, with maximum numbers occurring during spring, coinciding with the increase in the brood (Morgenthaler, 1939 and Weiser, 1961). In winter, spores are rarely found, or are only found in heavily infected bees. Any inherent natural defense by a bee colony against a heavy infection depends on the colony size and on the prevailing weather conditions during the early autumn of the previous year (Steche, 1985). If these conditions are unfavorable, the overall life span of the colony is reduced. This may lead to the premature death of bees during winter or early spring. In a typical case of a colony being depleted because of a Nosema infection, the queen can be observed surrounded by a few bees, confusedly attending to brood that is already sealed. In faecal droppings, spores may survive for more than one year (Bailey, 1957; 1967). Spores may also remain viable for up to four months after immersion in honey (White, 1919) and for up to 4.5 years in the cadavers of infected bees (Kulikov and Akramovsky, 1961). The spores may loss their viability after only 3 days when submerged in honey at hive temperature (Malone et al., 2001). It is likely that faecal contamination of wax, especially in combs used for brood rearing, or other hive interior surfaces, provides sufficient inoculums for N. apis to be easily transmitted to the next generation of bees. According to Iranian veterinary organization reports the apiaries' population is 413629 hives in study area. The aim of this study was to find out the ratio of Nosema infected honey bees in Urmia, North-West of Iran.

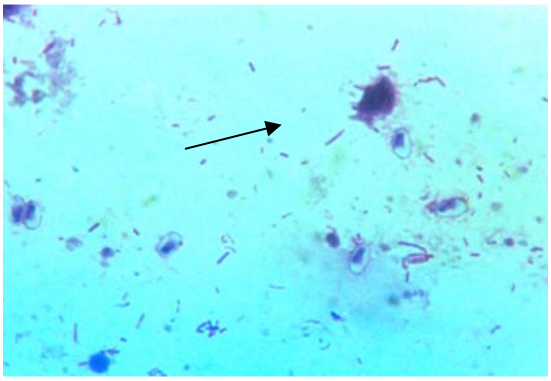


Figure 1: Spores of N.apis (staining with Giemsa stain) × 100

Materials and Methods

Source of samples

Between February and May 2002-2003, samples were randomly collected from 20 apiaries in Urmia region North-West of Iran. This region is semi-humid, with average annual rainfall of about 350 mm with the monthly average temperature of +28.3°C in August and -5°C in January. Sampling was done in four periods during 16 months from February 2002 to May 2003. In each period twenty collection points were sampled for *N. apis* infection. Two to twenty hives were sampled randomly from each apiary (Tables, 1 and 2). A total of 139, 133, 87 and 128 samples were taken from hives in February-May 2002 and February- May 2003, respectively.

Sample collection

Samples were collected directly from the hives in the morning. Samples were placed in a labeled container carefully; the name of the

region, date of sampling, type of hive (traditional or modern), hives population, numbers of sampling hives and the name of the owners were carefully recorded. At least 60 bees were collected in order to detect 5% of diseased bees with 95% confidence from each region (Fries, 1988).

Parasitological examination

All samples were transferred to the parasitology diagnostic laboratory of the Faculty of Veterinary Medicine, Urmia University. At least 60 bees were taken from each colony. A suspension of the abdomens of dead bees is prepared by grinding with 10 ml distilled water. The suspension was filtered via 100 µ mesh to remove debris that would interfere with the examination. To demonstrate *Nosema* spores, thin smears of suspension were prepared. When air-dried, ethanol-fixed smears of infected tissues (homogenates of the abdominal contents of affected bees) were stained with Giemsa stains (10% in 0.02 M phosphate buffer) for 45 minutes and examined microscopically at ×400 magnifications, according to (Cantwell, 1970) with slight simplification; this revealed the oval spores of *Nosema* with approximately 5–7 µm long and 3–4 µm wide (Fig.1). *N. apis* spores have a distinctive appearance, with thick unstained walls and an indistinct blue interior, without visible nuclei. Insect cells, fungal spores and other protozoa stained in this way generally have thinner walls, blue/purple cytoplasm and magenta-colored nuclei.

Table 1: Sampling area and result of infected hives to N. apis in the first period of study (February - May 2002) in Urmia region

	Winter 2002			Spring 2002		
	Numer of hives	Sample No.	Results	Numer of hives	Sample No.	Results
Urmia university farm	60	5	____	60	5	-
Garahagach	15	3	-	15	2	-
Emamzadeh	35	3	-	35	4	-
Silvana	100	7	-	100	8	-
Ziveh	50	5	-	50	4	-
Nazloo	105	8	-	105	10	-
Zinaloo	70	6	-	70	6	-
Tazehkand	115	10	-	115	10	-
Goeytapeh	84	5	-	84	5	-
Hajipirloo	97	4	-	97	5	-
Barandooz	65	5	-	65	4	-
Marmishoo	208	20	-	208	20	-
Rajan	70	5	-	70	5	-
Gasemloo	131	7	-	131	8	+
Dizaj_siavosh	36	4	-	36	3	-
Band	45	4	-	45	3	-
Neichelan	50	5	-	50	6	-
Khoshakoo	28	3	-	28	3	-
Mavanaa	80	8	-	80	10	-
Urmia	110	12	-	110	12	-
Total	1484	139	-	1484	133	-

Results

During this survey total of 487 hives were examined for infection with *Nosema apis*. In the first stage of study (February 2002), no infection was seen in sampled hives, but in second stage of same year (May 2002) the infection was seen in one apiary with 131 hives (Table 1). In third and forth stage of study (February and May 2003) the infection was seen in one and 4 apiaries, respectively (Table 2). In this study the infection rate in first year (< 10 spores in each microscopicfield) was lower than the second year (at least 10-50 spores in each field).

Table 2: Sampling area and result of infected hives to N.apis in the second period of study (February - May 2003) in Urmia region.

	Winter 2003			Spring 2003		
	Numer of hives	Sample No.	Results	Numer of hives	Sample No.	Results
Urmia university farm	60	4	-	60	5	
Zinaloo	120	8	-	120	9	
Zinaloo	100	5	-	100	9	+
Zinaloo	45	3	-	45	5	
Znaloo	6	1	-	6	1	
Band	10	2	-	10	2	
Band	60	4	-	60	5	
Seir	90	5	-	90	8	
Seir	4	1	-	4	1	
Seir	105	5	-	105	8	
Seir	110	6	-	110	8	
Nazloo	120	8	-	120	10	
Nazloo	10	2	-	10	1	
Nazloo	100	5	-	100	15	+
Nazloo	80	5	-	71	12	+
Nazloo	70	5	-	70	4	
Zonbalan	60	4	-	60	4	
Zonbalan	30	4	+	24	5	+
Zonbalan	70	5	-	70	8	
Zonbalan	90	5	-	90	8	
Total	8110	87		8095	128	

Discussion

The majority of Nosema-infected colonies will appear normal, with no obvious signs of disease even when the disease is sufficient to cause significant losses in honey production and pollination efficiency (Anderson & Giacon, 1992 and Goodwinet al., 1990). Any inherent natural defence by a bee colony against a heavy infection with the parasite depends on the colony size as well as on the prevailing weather conditions during the early part of the autumn of the previous year (Steche, 1985). If these conditions are unfavourable, the overall life expectancy of the colony is reduced. This may lead to the premature death of bees during winter or early spring. A proper diagnosis can be made only by microscopic examination of the adult bee ventriculus. *Nosema* spores must be differentiated from yeast cells, fungal spores, fat and calciferous bodies, and from *M. mellificae* cysts, which are spherical and approximately 6–7 µm in diameter.

The results showed the presence of infection to *Nosema* in Urmia and the low infection rate in the early months of first year (February - May 2002) of study that it was increased in next year. The reason of high infection rate in the second year of the study can be the result of more rainy and cloudy days in this period comparing to the first year. This has caused the hone bees to stay more often in the hives and increase spores excreted and subsequently spore intake were raised. Instead of meteorological organization of West Azerbaijan reports mean humidity and rainfall rate were increased and mean temperature rate decrease in 2003 in comparing with previous year.

The results of this study revealed that the infection of hives to *N.apis*. To prevent and control the infection the following remarks can be used. Spores may be killed by heating hive equipment or tools to a temperature of at least 60°C for 15 minutes. Combs may be sterilized by heating to 49°C for 24 hours (Cantwell and Shimanuki, 1970). Fumes from a solution of at least 60% acetic acid will inactivate any spores within a few hours, depending on the concentration; higher concentrations are even more effective and will kill spores within a few minutes (Bailey, 1957; De Ruiter, A. and Van der Steen, 1989).

In conclusion the result of this study showed low or moderate infection to *N. apis* in West Azarbaijan. However in the period of the study the fall peak of infection was not recorded in the region. In this situation effective control of *Nosema* disease is achieved by combining the sterilization of equipment using heat or acetic acid with fumagillin treatment.

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بررسی آلودگی به *نوزوما آپیس* در زنبورداری های ارومیه، شمال غرب ایران

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چکیده

نوزوما آپیس تک یاخته انگلی سلول‌های اپی تلیال دستگاه گوارش زنبوران بالغ می‌باشد و بیماری حاصل از آن در سراسر دنیا دیده می‌شود. این مطالعه در چهار پریود در بهمن ماه ۱۳۸۰ ، اردیبهشت و بهمن ۱۳۸۱ و اردیبهشت ۱۳۸۲ انجام پذیرفته است. در هر مرحله نمونه گیری از ۲۰ زنبورداری در شهرستان ارومیه و اطراف انجام شد. در اولین مرحله نمونه گیری آلودگی به انگل مشاهده نشد، در حالی که در مرحله دوم آلودگی فقط در یک زنبورداری مشخص گردید. در سومین و چهارمین مرحله نمونه گیری به ترتیب آلودگی در یک و چهار زنبورداری وجود داشت. نتایج نشاندهنده آلودگی بیشتر در سومین و چهارمین پریود نمونه گیری دارد، دلیل این امر افزایش روزهای ابری و بارانی در سال دوم نسبت به سال قبل بود. در چنین شرایطی زنبورها مدت بیشتری در کندو باقی می‌مانند، مدفوع بیشتری در کندو دفع می کنند و به علت تماس بیشتر زنبوران با یکدیگر بلع اسپور در موقع تمیز کردن شان ها بیشتر و آلودگی بیشتر صورت می‌پذیرد.

واژه‌های کلیدی: *نوزوما آپیس*، زنبورداری، زنبور عسل، ارومیه، ایران